FEBS Letters 580 (2006) 6391–6398

Site specific changes of redox metabolism in adipose tissue of obese Zucker rats

Anne Galinier[a,b,*], Audrey Carriere[a], Yvette Fernandez[a], Sylvie Caspar-Bauguil[a,b], Brigitte Periquet[b], Alain Periquet[a], Luc Penicaud[a], Louis Casteilla[a]

[a] *IFR 31, UMR 5018 CNRS-UPS, Bât L1, CHU Rangueil, TSA 50032, 31059 Toulouse Cedex 9, France*
[b] *Laboratoire de Biochimie, CHU Rangueil, 1 avenue Jean Poulhès, 31059 Toulouce Cedex 09, France*

Received 17 August 2006; revised 11 October 2006; accepted 24 October 2006

Available online 3 November 2006

Edited by Robert Barouki

Abstract **Adipose tissues are differently involved in lipid metabolism and obesity according to their type and location. Increasing reports stress on the impact of redox metabolism on obesity and metabolic syndrome. The aim of this work is to investigate the site-specific redox metabolism in three different adipose tissues and its changes occurring in obesity. We analysed enzymatic and non-enzymatic parameters, and focused on the reduced/oxidized glutathione and coenzyme Q couples. In lean compared with obese non-diabetic Zucker rats, interscapular brown fat seems well protected against oxidative stress and epididymal adipose tissue shows a more reduced glutathione redox state, associated with a higher susceptibility to lipophilic oxidative stress than inguinal adipose tissue. Epididymal adipose tissue redox metabolism significantly differs from inguinal one by its limited redox metabolism adaptation. Our results demonstrate site-specific managements of reactive oxygen species metabolism in obese Zucker rats. These results are not consistent with the classic deciphering of inflammatory situation and produce a new conception of the redox parameters implication in the development of the metabolic syndrome.**

Keywords: Site specificity; Adipose tissue; Obesity; Redox state; Glutathione; Coenzyme Q

1. Introduction

Adipose tissues play a key role in energy homeostasis and they function as lipid-storing (white adipose tissue, WAT) or energy-dissipating (brown adipose tissue, BAT) tissues. In parallel, adipose tissues act also as endocrine/paracrine organs, secreting a wide variety of molecules, adipokines and cytokines, which are involved in the development of morbid complications of obesity [1]. WAT is disseminated in different locations that display different metabolic properties and functions. This represents an important focus of current research due to the specific role that internal fat seems to play in metabolic disorders pathogenicity [2]. So, visceral abdominal obesity has been linked to the development of insulin resistance or type 2 diabetes mellitus and a cause–effect relationship has been established between internal fat and the metabolic syndrome [3–5]. Different results suggest that differences in adipocyte precursor pools and in the pattern of metabolism contribute to the dissimilar properties of the fat pads. These differences were extended to endocrine function and recently emphasized by gene expression profiling [6].

Reactive oxygen species (ROS) production and detoxification formed a set of oxido-reduction reactions named redox metabolism. Oxidative stress occurs when antioxidant defences are overwhelmed by an excessive ROS production. It has been implicated in the pathogenesis of several metabolic diseases as well as in the co-morbidity of diabetes mellitus [7] and atherosclerosis [8]. In such pathologies, redox metabolism was evaluated by the pattern of enzymatic and non-enzymatic cellular antioxidant defences such as superoxide dismutase (SOD), glutathione peroxidase (GPx) or catalase activities, and glutathione or α-tocopherol (α-Toc) contents in blood and liver [9,10]. Several cues seem also to relate obesity with pro-oxidative context and now lead to the emergent view characterizing obesity as a chronic inflammatory situation [11]. This disease is associated with an increase in the markers of systemic oxidative stress and its prevalence is correlated with decreased concentrations of plasma antioxidants [12]. Furthermore, it has been suggested that oxidative stress in accumulated fat is an early instigator of metabolic syndrome [13]. Nevertheless and in contrast, preadipocyte proliferation and differentiation are negatively controlled by ROS [14,15] while over expression of cellular GPx has been reported to promote obesity in mice [16]. One explanation of these apparent discrepancies could be raised from the fact that most of these studies were conducted not in situations of strict obesity but with confused metabolic pathologies defined as metabolic syndrome. Indeed, in a model of obesity with non-congruent metabolic disorders, inguinal adipose tissue displays a more intracellular reduced redox state which could promote on its own the development of a deleterious proadipogenic process [17]. However, no study investigates the comparative redox metabolism between different fat deposits in spite of their quite different pathogenicity in metabolic disorders.

The aim of this work was to compare the redox metabolism between interscapular brown adipose tissue (IBAT), inguinal and epididymal white fat pads (IWAT and EWAT, respectively) in lean and obese Zucker rats. This genetic rodent model of obesity is characterized by a defect in leptin signalling due to the absence of functional leptin receptor and is a

*Corresponding author. Address: Laboratoire de Biochimie, CHU Rangueil, 1 avenue Jean Poulhès, 31059 Toulouce Cedex 09, France. Fax: +33 5 61 32 36 90.
E-mail address: galinier.a@chu-toulouse.fr (A. Galinier).

0014-5793/$32.00
doi:10.1016/j.febslet.2006.10.052

non-diabetic obese model. The two white fat pads were chosen, respectively, as subcutaneous and internal adipose tissue because they represent the main lipid storages sites of rat [18]. As we previously shown that the mainly of antioxidant molecules was in adipocytes versus stroma vascular fraction of adipose tissue [17], the measure of these molecules contents was executed in whole adipose tissue.

2. Materials and methods

2.1. Animals

Obese and lean Zucker rats (11 weeks old) were purchased from Charles River Laboratories, L'Arbresle, France. They were acclimated 7 days to 22 ± 1 °C in individual cage, kept under intermittent 12 h periods of light and dark, and given commercial food (2016 -Harlan, Gannat, France) and water ad libitum. Animals were weighted and then anesthetized with 100 mg ketamine and 10 mg xylazine/kg body weight.

2.2. Tissues isolation

The different fat pads were rapidly removed after rat's exsanguination. IBAT, IWAT and EWAT were carefully dissected to remove adhering tissues as capillaries, ganglions and contaminating WAT (surrounding the IBAT). Tissues were washed in phosphate buffered saline medium, weighted and immediately frozen in liquid nitrogen. Prior to measurements, aliquots were pulverized with a stainless steel mortar and pestle. Powders were stored at −80 °C until use.

2.3. Samples preparation

One hundred milligrams of each powered frozen tissue was used for quinines measurement while the left was homogenized in 3 mM EDTA, 154 mM KCl, pH 7.4 (1:10 wt/vol for IBAT, 1:2 wt/vol for IWAT and EWAT). Five hundred microlitres of this homogenate was used for analysis of cytochrome *c* oxydase (Cytox) and catalase activities and glutathione and vitamin C contents. The left homogenate was sonicated (30 s, 4 °C) and then centrifuged at 8000 × *g* for 10 min, delipided and again centrifuged at 105 000 × *g* for 45 min (all these steps were performed at 4 °C). Supernatant was used for measures of antioxidant SOD and GPx activities.

2.4. Enzymatic activities

Protein assays were performed in the homogenate and the supernatant [19]. SOD activities (Mn SOD, Cu/Zn SOD) were assayed by using the inhibition of pyrogallol autoxidation [20]. One unit (U) of SOD activity was defined as the amount of enzyme that inhibited pyrogallol autoxidation by 50%. Catalase activity was determined by measuring decomposition of H_2O_2 at 240 nm [21]. Glutathione peroxidase seleno-dependant (GPx) activity was measured using *t*-butylhydroperoxide as substrate [22]. One unit of GPx activity corresponds to 1 μmole of NADPH oxidized per minute. Mitochondrial enzymatic activity (Cytox) was assayed by measurement of cytochrome *c* oxidation as previously described [23].

2.5. α-Toc, CoQ9, CoQ10, glutathione and vitamin C (VitC)

Powdered frozen tissues (100 mg) were mixed and extracted in 2-propanol, to detect CoQs (Q9 and Q10) in their oxidized and reduced forms and α-Toc by reverse-phase HPLC with electrochemical detection, on the same run as previously described [24]. Results were expressed as nmol/g tissue because these lipophilic molecules mainly interact with other lipophilic component and that fat pad weight is directly correlated with its lipid content. Fifty microlitres of tissular homogenates was immediately mixed with 500 μl of 4% metaphosphoric acid in H_2O/methanol (3:1 v/v) or with 450 μl of 1% EDTA/5% metaphosphoric acid (1:5 v/v), respectively, for vitamin C and glutathione measure. After centrifugation (3000 × *g*, 10 min, 4 °C), supernatants were used to detect reduced glutathione (GSH) and its oxidized form (GSSG), total VitC and its oxidized form dehydroascorbic acid (DHAA) by reverse-phase HPLC with electrochemical detection [25] and fluorimetric detection [26], respectively. The results were expressed as μmol/g tissular proteins. Total glutathione, named "equivalent glutathione" (Eq GSH), was the sum of GSH and two fold GSSG concentrations (2GSH → 1GSSG).

We calculated the redox state of glutathione, VitC and CoQs as ([oxidized form]/[total forms]) × 100 with [total forms] = [oxidized form] + [reduced form].

2.6. Malondialdehyde (MDA)

Lipid peroxidation in homogenate was estimated from the formation of the MDA by a spectrofluorimetric method [27].

2.7. Statistical analysis

Means ± S.E.M. were calculated and statistically significant differences between two groups were determined by Student's *t*-test at $P < 0.05$.

3. Results

3.1. Comparison of redox metabolism in different fat pads of lean Zucker rats

The weight of IBAT, IWAT and EWAT of 12 lean rats were, respectively, 0.34 ± 0.07 g, 3.44 ± 0.61 g and 1.79 ± 0.48 g.

Fig. 1 showed the different antioxidant enzymatic defences and the Cytox activity used as an index of mitochondrial oxidative potential. As expected, IBAT had the highest Cytox and Mn-SOD activities. The lowest activities of Cytox and Mn-SOD were observed in IWAT and EWAT respectively. No significant difference can be observed in Cu/Zn-SOD between all deposits. Catalase and GPx activities were the highest in EWAT. To propose an integrate view of antioxidant enzymatic metabolism, we calculated different ratios. As tot SOD activity generates H_2O_2 that is destroyed by Catalase and GPx (the latter is also able to destroy other hydroperoxides), the ratios of Cat/tot SOD and GPx/tot SOD activities are taken as indexes of cellular H_2O_2 metabolism. However, as mitochondria is the major site of ROS generation in cells [28], and that increase in $O_2^{\cdot-}$ is reflected by SOD synthesis and activity [29], the ratio of Mn-SOD/Cytox activities can be considered as index of mitochondrial $O_2^{\cdot-}$ metabolism. The values of these different ratios were given in Table 1. IBAT had the lowest ratio of GPx/tot SOD activities whereas the values of the Cat/tot SOD and GPx/tot SOD ratios were significantly higher in EWAT than in IWAT. In contrast, the ratio Mn-SOD/Cytox was higher in IWAT than in EWAT.

Concerning the hydrophilic molecules, IBAT was significantly different from both WAT together by a lower glutathione redox state, a higher VitC redox state and a lower VitC content (Fig. 2A). In contrast, the content of lipophilic molecules, CoQ9, CoQ10 and α-Toc, were the highest in IBAT (Fig. 2A and B). Redox states of CoQs were similar in all fat tissues. EWAT significantly distinguished from IWAT by a lower glutathione redox state, a higher equivalent glutathione and VitC content, a lower CoQ10 content (Fig. 2A) and a higher MDA content (Fig. 2C).

3.2. Changes of redox metabolism in different fat pads of obese Zucker rats

The weight of IBAT, IWAT and EWAT of seven obese rats were, respectively, 0.67 ± 0.08 g, 18.14 ± 4.11 g and 3.85 ± 1.14 g.

For IBAT, Cytox as GPx activities were significantly reduced in obese Zucker comparatively to lean whereas Cat

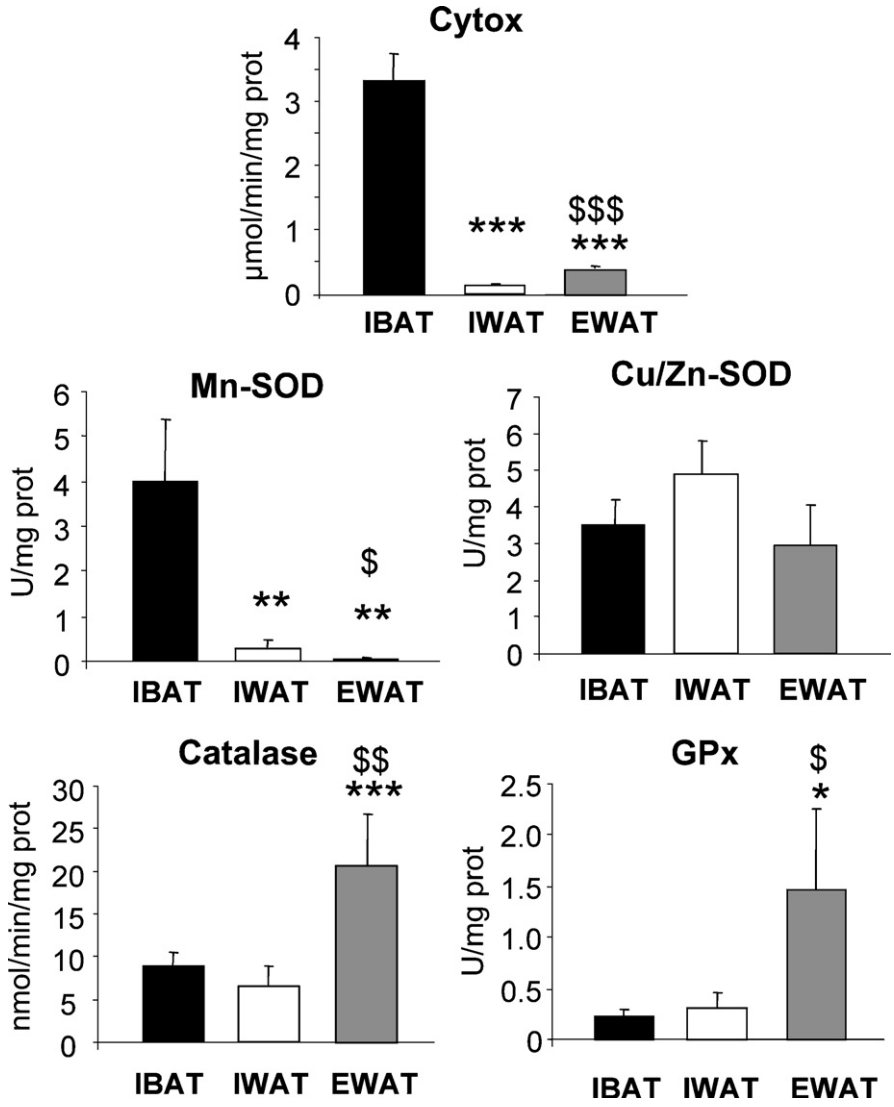

Fig. 1. Enzymatic activities in adipose tissues of lean Zucker rats. Interscapular brown adipose tissue (IBAT), inguinal (IWAT) and epididymal (EWAT) white adipose tissues. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ IWAT or EWAT versus IBAT; $\$P < 0.05$, $\$\$P < 0.01$, $\$\$\$P < 0.001$ IWAT versus EWAT.

Table 1
Enzymatic activities ratio in adipose tissues of lean and obese rats

	Cytosol H_2O_2 metabolism		Mitochondria O_2^{-} metabolism
	Cat/tot SOD $\times 10^{-3}$	GPx/tot SOD	MnSOD/Cytox
IBAT			
Lean	1.02 ± 0.03	26.49 ± 10.26	1.394 ± 0.090
Obese	2.96 ± 0.26[a]	12.90 ± 3.55	1.603 ± 0.106
IWAT			
Lean	1.34 ± 0.18[c]	55.26 ± 9.25[b]	3.221 ± 0.908[c]
Obese	4.20 ± 0.81[a]	97.95 ± 29.98[b]	1.897 ± 0.458
EWAT			
Lean	10.49 ± 3.04[b]	807.87 ± 373.69[b]	0.096 ± 0.096[b]
Obese	4.27 ± 0.93	81.04 ± 19.13[a,b]	1.731 ± 0.274[a]

Interscapular brown adipose tissue (IBAT), inguinal (IWAT) and epididymal (EWAT) white adipose tissues.
[a] $P < 0.05$ obese versus lean.
[b] $P < 0.05$ IWAT or EWAT versus IBAT.
[c] $P < 0.05$ IWAT versus EWAT.

activity was increased (Fig. 3). Cytox and Cat were significantly increased in IWAT whereas GPx was decreased in EWAT. The ratio of Cat/tot SOD activities was significantly higher in IBAT as for IWAT in obese compared to lean rats when GPx/tot SOD was intensely lower in EWAT (Table 1). The ratio Mn SOD/Cytox was only significantly increased in EWAT between lean and obese. About the hydrophilic molecules, the two WAT of obese differed from IBAT by an increase in VitC content associated with a decrease in its redox state, comparatively to lean (Fig. 4A). IBAT glutathione redox states were very heterogeneous and seemed increased in obese but the difference was not significant. On the contrary, the glutathione redox state was decreased in WAT but only significantly in IWAT (Fig. 4A). The three lipophilic molecules contents (CoQ9, CoQ10 and α-Toc) were differently altered in IBAT, IWAT and EWAT of obese rats (Fig. 4A and B). In IBAT, CoQ9 and CoQ10 contents were decreased and the CoQ10 redox state was increased. The three molecules contents were significantly decreased in IWAT, whereas only

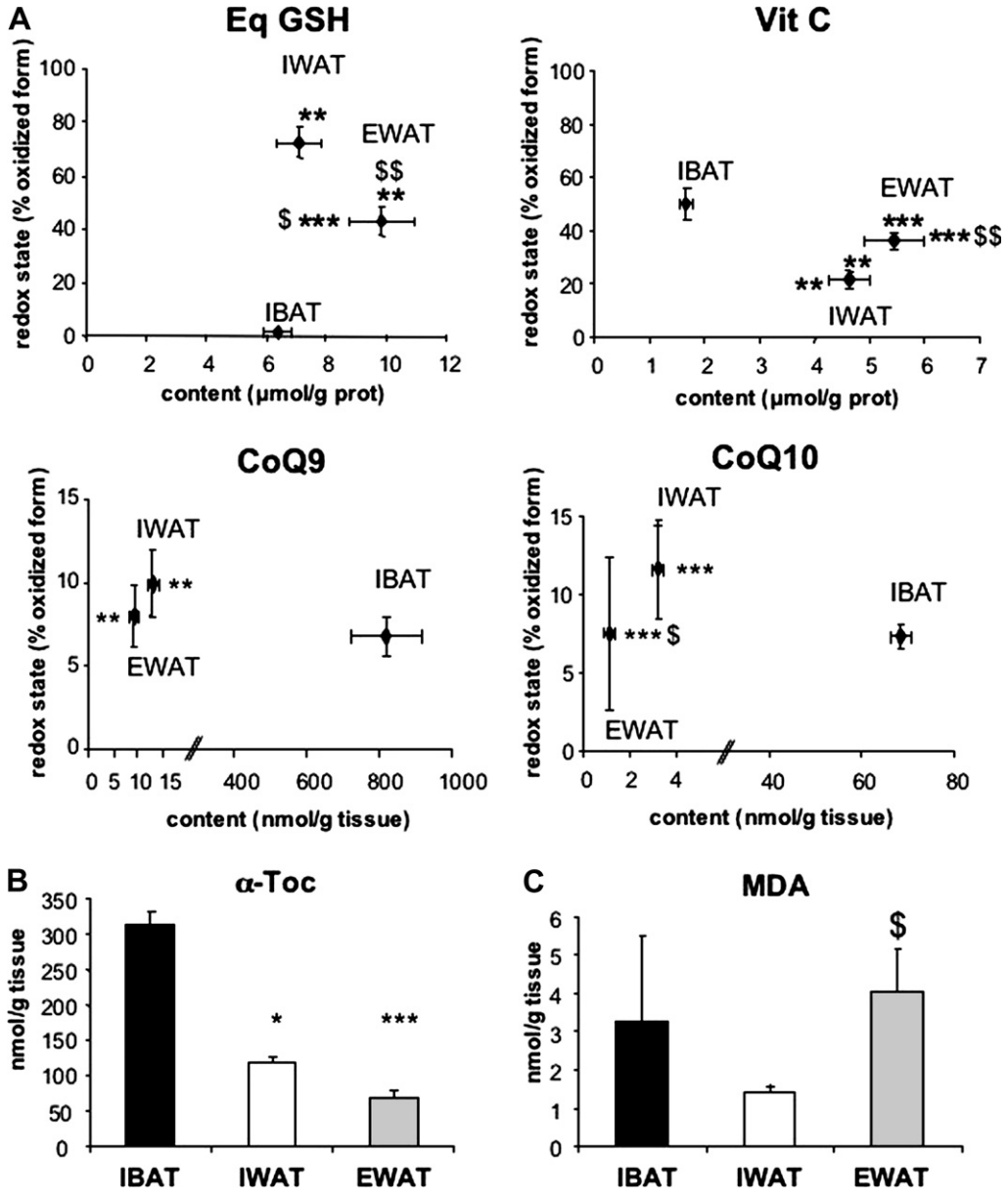

Fig. 2. Antioxidant molecules and lipid peroxidation in adipose tissues of lean Zucker rats. Interscapular brown adipose tissue (IBAT), inguinal (IWAT) and epididymal (EWAT) white adipose tissues. (A) Equivalent glutathione (Eq GSH), vitamin C (VitC), CoQ9 and CoQ10 status: redox state versus content. (B) α-tocopherol (α-Toc) content. (C) MDA content. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ IWAT or EWAT versus IBAT; $\$P < 0.05$, $\$\$P < 0.01$ IWAT versus EWAT.

α-Toc content was decreased in EWAT. MDA content was decreased in the two WAT but not in IBAT (Fig. 4C).

4. Discussion

Recently, an excessive or sustained increase in ROS production has been implicated in the pathogeneses of several diseases, as well as in the co-morbidity of atherosclerosis and diabetes mellitus associated with obesity [7,8]. Here, we demonstrate that redox metabolism of fat tissues is different according to the location of the deposit. Surprisingly, obesity induced a more reduced state in the WAT whatever the deposit but with site-specific changes in subcutaneous and epididymal fats.

Redox metabolism involves multiple interdependent enzymatic and non-enzymatic molecules, which can counteract ROS and prevent oxidative stress. In addition with the different molecule contents and enzymatic activities, we calculated different ratios to have an integrated view of such metabolism.

Altogether our results demonstrated that in lean Zucker rats, the profiles of enzymatic and non-enzymatic defences are different in the three fat pads. The IBAT thermogenic metabolism was highly linked to its mitochondria content and activity. As expected [23], the mitochondrial oxidative potential (Cytox activity) was particularly high in IBAT of these rats suggesting a high mitochondrial ROS production. Moreover, the high level of coenzymes Q in IBAT is consistent first, with the necessary intensive respiratory mitochondrial chain activity which finally enhanced the function of UCP1 and second,

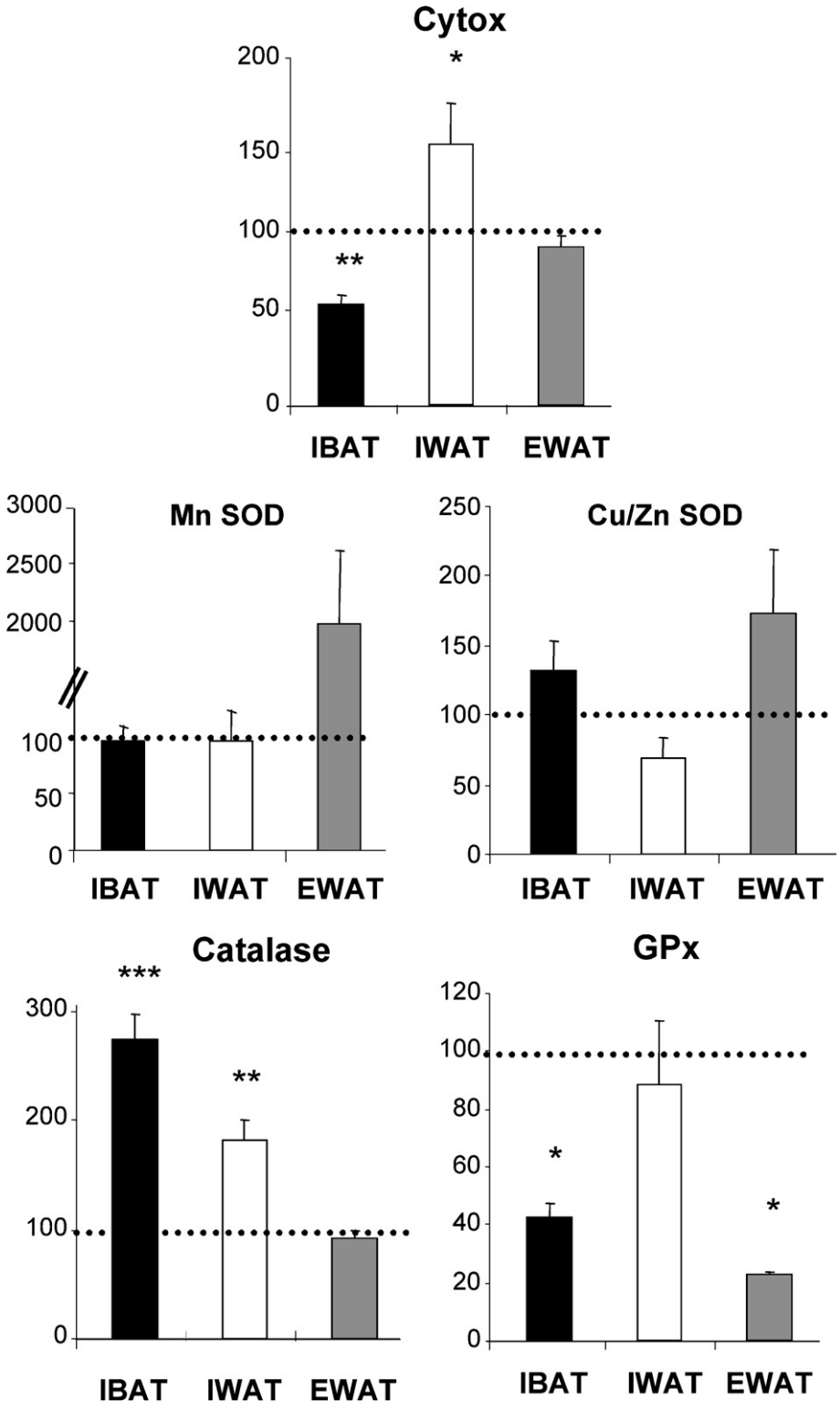

Fig. 3. Enzymatic activities in adipose tissues of obese Zucker rats. Values were expressed as the % of the lean values for interscapular brown adipose tissue (IBAT), inguinal (IWAT) and epididymal (EWAT) white adipose tissues. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus lean.

with the function of the UCP1 itself [30]. IBAT glutathione was entirely in the reduced state as it was described for liver [31] that indicates the absence of oxidative stress in the cellular hydrophilic compartment. This could be explained by the high UCP1 expression in IBAT mitochondria, which can decrease the mitochondrial ROS generation [32]. Moreover, IBAT seems well protected against excessive oxidative aggression according to its high content in lipophilic antioxidants. In subcutaneous and epididymal WAT, the contrasted ROS metabolism profiles seemed to be linked with the lipid metabolism. In

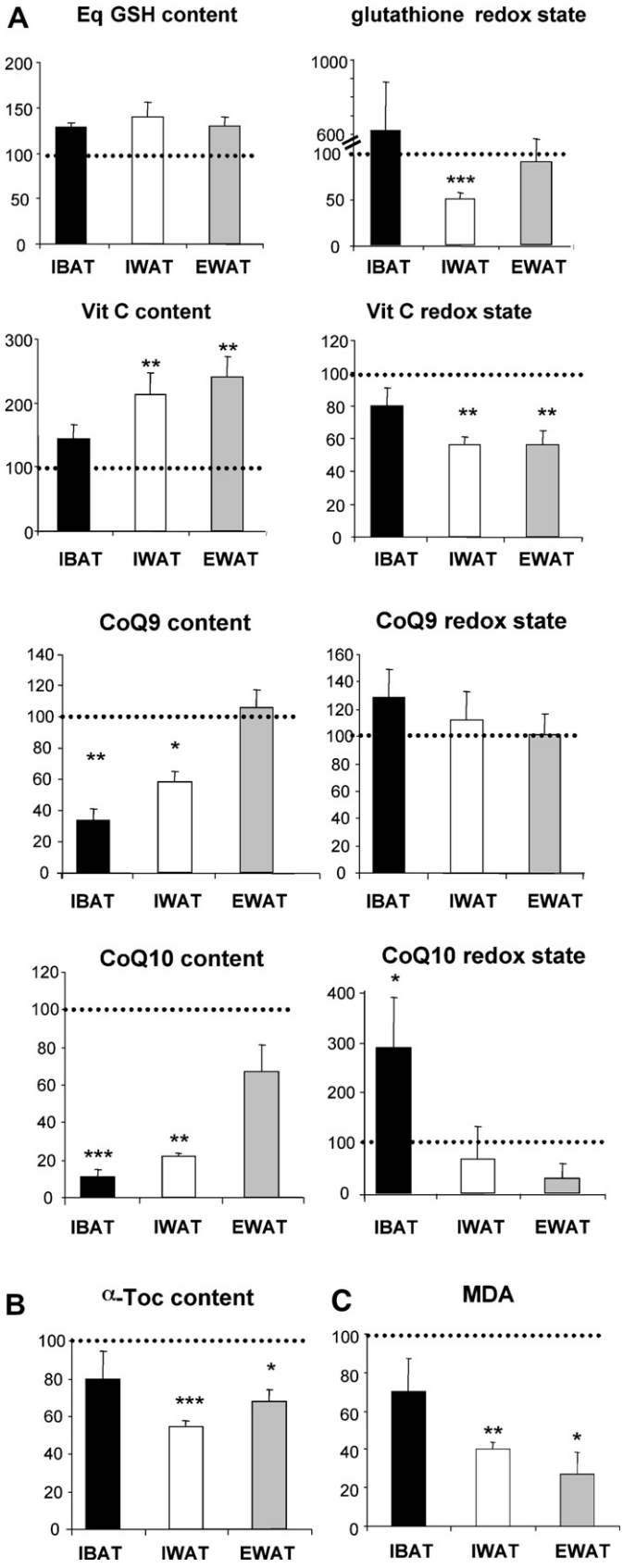

Fig. 4. Antioxidant molecules and lipid peroxidation in adipose tissues of obese Zucker rats. Values were expressed as the % of the lean values for interscapular brown adipose tissue (IBAT), inguinal (IWAT) and epididymal (EWAT) white adipose tissues. (A) Equivalent glutathione (Eq GSH), vitamin C (VitC), CoQ9 and CoQ10 status: content and redox state. (B) α-tocopherol (α-Toc) content. (C) MDA content. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus lean.

lean, glutathione redox state was lower in epididymal WAT than in subcutaneous one (43% versus 72%) and indicated a more reduced state in hydrophilic compartments of epididymal WAT. As we have previously described that a positive link between reduced glutathione and lipid storage exists in adipocyte [17], glutathione metabolism could reflect the more intense lipid storage displayed by epididymal fat in lean animals. Indeed, Di Girolamo et al. [18], showed that the cumulative growth of EWAT was due mostly to hypertrophy (identified by the cellular triglyceride content) whereas the growth of IWAT was due predominantly to apparent hyperplasia. Finally, MDA content could indicate a higher oxidative stress in lipophilic compartment of epididymal WAT. Although the ratios of Cat/tot SOD and GPX/tot SOD activities indicate that epididymal WAT is the most efficient to eliminate H_2O_2 potentially produced. This could be attributed to a higher susceptibility to lipophilic and mitochondrial oxidative stress reflected by the lower lipophilic antioxidant contents (mostly CoQ10) and the lower ratio MnSOD/Cytox in epididymal WAT comparatively to subcutaneous one.

Thus the redox metabolism in fat pads indicates and promotes site-specific metabolism. Its importance must be interpreted in favour of the mitochondrial needs for the IBAT and the lipogenesis set up in WATs.

This led us to investigate the changes occurring in obesity. To delineate the obesity effect *per se* from other congruent metabolic disorders often associated in animal model, we chose to perform our experiments on 11 weeks old Zucker rats. Indeed, we previously demonstrated that such animals are obese and glucose intolerant without diabetic symptoms [17]. Although we used a genetically obese Zucker rat in this study, this model can be considered as relevant with the redox metabolism in human adipose tissue. Indeed, although leptin is known to induce oxidative stress, most of obese subjects are unresponsive to lipostatic action of leptin because its receptor gradually disappeared in adipose tissue during obesity [33]. Then, we can hypothesize that leptin dependant signaling pathways are poorly functional to induce oxidative stress in adipose tissue of obese individuals. In such situation, important enzymatic and non-enzymatic alterations appeared in the three fat pads of obese rats comparatively to lean ones. Enzymatic activities were essentially altered in obese IBAT. This tissue has a mitochondrial oxidative metabolism reduced associated to a decrease in UCP1 expression. The lower oxidative metabolism and the higher ratio of Cat/tot SOD activities are in accordance with the lack of changes in glutathione and VitC redox states in obese comparatively to lean IBAT. This last result indicates no alteration of the reduced state of hydrophilic compartment of this fat tissue in obese phenotype. Moreover, the important decrease in CoQs content of obese IBAT was without consequence on the MDA content probably by sufficient α-Toc content. Altogether, this demonstrates that in spite of the dramatic transformation of IBAT into WAT-like fat, IBAT maintains its specific redox status and is preserved from oxidative stress during obesity.

As previously reported, subcutaneous WAT shows a more reduced state in obese animal than in the control one [17]. The decrease in VitC and glutathione redox states indicates a higher reduced environment in hydrophilic sites. Surprisingly, these changes are associated with an increase in VitC content. This needs to be investigated further because no report relates any link between adipose tissue VitC metabolism and obesity.

Fig. 5. Index site specific weight (fat pad weight/total weight) and redox changes during obesity in inguinal (IWAT) and epididymal (EWAT) white adipose tissues.

The decrease of lipophilic oxidative stress, as revealed by the decrease in MDA content in both WAT deposits, is consistent with the increase of the reduced state in the hydrophilic compartment. Indeed, over expression of GPx [16] as well as of glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the maintenance of redox potential via production of NADPH (substratum for GSH regeneration) [34] or different antioxidants [17] stimulate adipogenesis of cultured preadipocytes. A similar trend can be also observed in epididymal WAT in spite of fewer changes suggesting that this tissue is close to an "already hypertrophic situation" in lean rats. This conclusion has to be paralleled with the ratio of fat pad weights (obese/lean) for inguinal and epididymal location (approximately 6 versus 1.5, respectively). Considering the enzymatic activities in WAT, it is noteworthy that, comparatively to lean, the mitochondrial oxidative potential was only increased in obese subcutaneous WAT with a concomitant decreased of CoQ, whereas indexes of the ability to eliminate ROS was increased in subcutaneous WAT but decreased in epididymal WAT. Altogether, these results indicate that during obesity, subcutaneous fat appears able to extensively store lipids whereas this metabolic pathway is confined in epididymal fat pad (Fig. 5). On the other hand subcutaneous WAT can adapt its redox metabolism whereas the capability of epididymal one to metabolize cytoplasm H_2O_2 and other peroxides seems to be limited. Then, the modulated intracellular ROS in these fat pads could differently trigger stress intracellular signalling pathways which in turn could participate to the deleterious effect of epididymal fat enlargement [35].

Taken together, these specific changes of redox metabolism in epididymal and subcutaneous adipose tissues are not consistent with the classic view of inflammatory situation. Then, they open a new conception of the redox parameters implication in the development of obesity and its link with metabolic syndrome.

References

[1] Rajala, M.W. and Scherer, P.E. (2003) Minireview: the adipocyte-at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144, 3765–3773.
[2] Bjorntorp, P. (1996) The regulation of adipose tissue distribution in humans. Int. J. Obes. Relat. Metab. Disord. 20, 291–302.
[3] Ascaso, J.F., Romero, P., Real, J.T., Lorente, R.I., Martinez-Valls, J. and Carmena, R. (2003) Abdominal obesity, insulin resistance, and metabolic syndrome in a southern European population. Eur. J. Inter. Med. 14, 101–106.

[4] Wajchenberg, B.L. (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr. Rev. 21, 697–738.
[5] Giusti, V., Suter, M., Verdumo, C., Gaillard, R.C., Burckhardt, P. and Pralong, F.P. (2004) Molecular determinants of human adipose tissue: differences between visceral and subcutaneous compartments in obese women. J. Clin. Endocrinol. Metab. 89, 1379–1384.
[6] Atzmon, G., Yang, X.M., Muzumdar, R., Ma, X.H., Gabriely, I. and Barzilai, N. (2002) Differential gene expression between visceral and subcutaneous fat depots. Horm. Metab. Res. 34, 622–628.
[7] Faure, P., Ramon, O., Favier, A. and Halimi, S. (2004) Selenium supplementation decreases nuclear factor-kappa B activity in peripheral blood mononuclear cells from type 2 diabetic patients. Eur. J. Clin. Invest. 34, 475–481.
[8] Touyz, R.M. and Schiffrin, E.L. (2004) Reactive oxygen species in vascular biology: implications in hypertension. Histochem. Cell. Biol. 122, 339–352.
[9] Ford, E.S., Mokdad, A.H., Giles, W.H. and Brown, D.W. (2003) The metabolic syndrome and antioxidant concentrations. Diabetes 52, 2346–2352.
[10] Yang, S., Zhu, H., Li, Y., Lin, H., Gabrielson, K., Trush, M.A. and Diehl, A.M. (2000) Mitochondrial adaptations to obesity-related oxidant stress. Arch. Biochem. Biophys. 378, 259–268.
[11] Dandona, P., Aljada, A. and Bandyopadhyay, A. (2004) Inflammation: the link between insulin resistance, obesity and diabetes. Trend Immunol. 25, 4–7.
[12] Reitman, A., Friedrich, I., Ben-Amotz, A. and Levy, Y. (2002) Low plasma antioxidants and normal plasma B vitamins and homocysteine in patients with severe obesity. Isr. Med. Assoc. J. 4, 590–593.
[13] Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M. and Shimomura, I. (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. J. Clin. Invest. 114, 1752–1761.
[14] Carrière, A., Fernandez, Y., Rigoulet, M., Pénicaud, L. and Casteilla, L. (2003) Inhibition of preadipocyte proliferation by mitochondrial reactive oxygen species. FEBS Lett. 550, 163–167.
[15] Carrière, A., Carmona, M.C., Fernandez, Y., Rigoulet, M., Wenger, R.H., Pénicaud, L. and Casteilla, L. (2004) Mitochondrial reactive oxygen species control the transcription factor CHOP-10/GADD153 and adipocyte differentiation. J. Biol. Chem. 279, 40462–40469.
[16] McClung, J.P., Roneker, C.A., Mu, W., Lisk, D.J., Langlais, P., Liu, F. and Lei, X.G. (2002) Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc. Natl. Acad. Sci. USA 101, 8852–8857.
[17] Galinier, A., Carrière, A., Fernandez, Y., Carpéné, C., André, M., Caspar-Bauguil, S., Thouvenot, J.P., Périquet, B., Pénicaud, L. and Casteilla, L. (2006) Adipose tissue pro-adipogenic redox changes in obesity. J. Biol. Chem. 281, 12682–12687.
[18] Di Girolamo, M., Fine, J.B., Tagra, K. and Rossmanith, R. (1998) Qualitative regional differences in adipose tissue growth and cellularity in male Wistar rats fed ad libitum. Am. J. Physiol. 274, R1460–R1467.
[19] Lowry, O.H., Rosebrough, N.J., Farr, A. and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
[20] Marklund, S. and Marklund, G. (1974) Involvement of the superoxyde anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.
[21] Clairbone, A.L. (1985) in: Handbook of Methods for Oxygen Radical Research (Grenwald, R.A., Ed.), CRC Press.
[22] Günzler, W.A., Kremers, H. and Flohe, L. (1974) An improved coupled test procedure for glutathione peroxidase in blood. Z. Klin. Chem. Klin. Biochem. 12, 444–448.
[23] Prunet-Marcassus, B., Moulin, K., Carmona, M.C., Villarroya, F., Penicaud, L. and Casteilla, L. (1999) Inverse distribution of uncoupling proteins expression and oxidative capacity in mature adipocytes and stroma-vascular fractions of rat white and brown adipose tissues. FEBS Lett. 464, 184–188.
[24] Galinier, A., Carrière, A., Fernandez, Y., Bessac, A.M., Caspar-Bauguil, S., Périquet, B., Comtat, M., Thouvenot, J.P. and Casteilla, L. (2004) Biological validation of coenzyme Q redox state by HPLC-EC measurement: relationship between coezyme Q redox state and coenzyme Q content in rat tissues. FEBS Lett. 578, 53–57.
[25] Melnyk, S., Pogribna, M., Pogribny, I., Hine, R.J. and James, S.J. (1999) A new HPLC method for the simultaneous determination of oxidized and reduced plasma aminothiols using coulometric electrochemical detection. J. Nutr. Biochem. 10, 490–497.
[26] Speek, A.J., Schrijver, J. and Schreurs, W.H. (1984) Fluorimetric determination of total vitamin C in whole blood by high-performance liquid chromatography with pre-column derivatization. J. Chromatogr. 305, 53–60.
[27] Yagi, K. (1984) Lipid peroxidation. Assay for blood plasma or serum. Method Enzymol. 105, 328–333.
[28] Turrens, J.F. (2003) Mitochondrial formation of reactive oxygen species. J. Physiol. 552, 335–344.
[29] Hassan, H.M. and Fridovich, I. (1979) Intracellular production of superoxide radical and hydrogen peroxide by redox active compounds. Arch. Biochem. Biophys. 196, 385–395.
[30] Echtay, K.S., Winkler, E. and Klingerberg, M. (2000) Coenzyme Q is an obligatory cofactor for uncoupling protein function. Nature 408, 609–613.
[31] Harvey, P.R., Ilson, R.G. and Strasberg, S.M. (1989) The simultaneous determination of oxidized and reduced glutathiones in liver tissue by ion pairing reverse phase high performance liquid chromatography with a coulometric electrochemical detector. Clin. Chim. Acta 180, 203–212.
[32] Nègre-Salvayre, A., Hirtz, C., Carrera, G., Cazenave, R., Troly, M., Salvayre, R., Penicaud, L. and Casteilla, L. (1997) A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. FASEB J. 11, 809–815.
[33] Wang, M.Y., Orci, L., Ravazzola, M. and Unger, R.H. (2005) Fat storage in adipocytes requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. PNAS 102, 18011–18016.
[34] Park, J., Rho, H.K., Kim, K.H., Choe, S.S., Lee, Y.S. and Kim, J.B. (2005) Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. Mol. Cell. Biol. 25, 5146–5157.
[35] Schafer, F.Q. and Buettner, G.R. (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Rad. Biol. Med. 30, 1191–1212.